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Inhibition of passive sensitization of human peripheral basophils by synthetic human immunoglobulin E peptide fragments

Noriki Nio[a], Katsuya Seguro[a], Yasuo Ariyoshi[a], Kiyomi Imano[b], Ikuhisa Yakuo[b], Atsuko Kita[b] and Hideo Nakamura[b]

[a]*Central Research Laboratories, Ajinomoto Co., 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki 210, Japan* and [b]*Research Laboratories, Dainippon Pharmaceutical Co., 33-93 Enoki, Suita, Osaka 564, Japan*

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To delineate the binding site in the human immunoglobulin E (IgE) molecule to the Fcε receptor on basophils and mast cells, we chemically synthesized a total of 71 peptide fragments within the sequence Ser^{300}–Lys^{547} in the human IgE molecule. The synthetic peptides were tested for their capacity to inhibit passive sensitization of human peripheral basophils with atopic patient's serum containing the specific IgE against dust mites in vitro. It was found that a peptide fragment, Pro^{345}–Ile^{356}, potently inhibited the passive sensitization. To clarify the minimal active core, various analogues, such as shortened, substituted (by Gly or Ala residue), omission and retro-sequence peptides, were synthesized and assayed. The results suggested that the sequence Pro^{345}–Lys^{352} in the human IgE molecule would be an IgE binding site, and that a synthetic octapeptide, Pro^{345}-Phe-Asp-Leu-Phe-Ile-Arg-Lys^{352}, inhibited the passive sensitization, probably by occupying the Fcε receptor sites on the cells.

Immunoglobulin E; IgE; Passive sensitization; Basophil; Histamine; FcεRI

1. INTRODUCTION

Immunoglobulin E (IgE) mediates immediate hypersensitization such as asthma, hay fever, food and drug allergies. IgE circulates in the blood and binds to the Fcε-chain type I receptor (FcεRI) on the surfaces of mast cells and basophils [1]. Bridging the receptor-bound IgE by a specific multivalent antigen triggers secretion of chemical mediators such as histamine, slow-reacting substance of anaphylaxis and platelet activating factor, which are responsible for type I immediate hypersensitivity [2,3]. It was thought that, by binding to and blocking the FcεRI, certain peptides including the binding site(s) would inhibit release of the chemical mediators. Such an IgE receptor-binding peptide will become an anti-allergic agent for type I immediate hypersensitivity [4].

Several approaches have been utilized to determine the binding site to the human FcεRI. Studies with proteolytic peptide fragments of human IgE [5], as well as conformational studies [6] after gentle heating have suggested that the binding site lies in the Fcε region, in particular, in the CH3 and CH4 domains. Recombinant Fcε-chain fragments within two-third of the CH2, and all of the CH3 and CH4 domains have blocked passive sensitization of human skin with IgE [7–9]. Therefore, it has been suggested that the binding site would be located in the CH3 and/or CH4 domains. However, the exact binding sites remain unidentified.

We previously reported that synthetic human IgE peptide fragments with inhibitory activity on passive cutaneous anaphylaxis (PCA) in rats were located within the sequences Ala^{329}–Thr^{357} in the CH3 domain, and Arg^{419}–Ala^{463} in the junction between the CH3 and CH4 domains, and that these two regions might be responsible for binding of IgE to its receptor [10]. However, it has been suggested that the interaction between IgE and its receptor is relatively specific to species [11]. In this study, the synthetic human IgE peptide fragments were tested for inhibitory activity of passive sensitization of human peripheral basophils with atopic patient's serum (allergen specific human IgE) in vitro. On the other hand, it has recently been suggested that the FcεRI-binding site on human IgE is located within the sequence Gln^{301}–Arg^{376} in the junction between the CH2 and CH3 domains [12]. To pinpoint the binding site more precisely, we continued the synthesis of peptide fragments within the sequence Ser^{300}–Lys^{547} corresponding to the C-terminus in the human IgE molecule. This paper describes the inhibition of the passive sensitization by synthetic human IgE peptide fragments.

Correspondence address: N. Nio, Central Research Laboratories, Ajinomoto Co., 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki 210, Japan. Fax: (81) (44) 244 9617; H. Nakamura, Research Laboratories, Dainippon Pharmaceutical Co., 33-93 Enoki, Suita, Osaka 564, Japan. Fax: (81) (6) 338 7656.

Abbreviations: These follow the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature in Eur. J. Biochem., 138, 9–39 (1984).

2. MATERIALS AND METHODS

2.1. *Peptide synthesis*

All peptides used were synthesized by the stepwise solid-phase method using Fmoc chemistry [12,13] as described previously [15,16]. The purity of each peptide was confirmed by thin-layer chromatography, high-performance liquid chromatography, fast atom bombardment mass spectrometry, sequencing with a gas-phase microsequencer, and amino acid analysis after hydrolyzing in constant-boiling HCl at 110°C for 24 h. The sequences of compounds are shown in Tables I–IV.

2.2. *Inhibitory activity of the synthetic peptides*

The ability of the synthesized peptides to passive sensitization of human peripheral basophils with allergen-specific human IgE in vitro was assayed by the method of Nakamura et al. [17]. A solution consisting of 120 mM NaCl, 5 mM KCl, 0.03% human serum albumin, and 25 mM Tris was adjusted to pH 7.6. This solution was used as a standard buffer for the assay. The passive sensitization was carried out according to the modified method of Pruzansky et al. [18]. Peripheral basophils (leukocyte fraction) were separated from whole blood obtained from healthy volunteers. The blood (50 ml) was mixed with 0.1 M EDTA (4 ml) and 6% dextran/3% glucose in physiological saline (12.5 ml), and allowed to sediment at room temperature for 60 min. The upper layer was centrifuged at 1,200 rpm for 10 min. The precipitate containing the leukocyte fraction was washed once with the standard buffer containing 4 mM EDTA, incubated with lactate buffer (pH 3.9) at 0°C for 5 min, washed twice with the standard buffer containing 4 mM EDTA and the cells were suspended in the standard buffer containing 4 mM EDTA and heparin (12.5 μg/ml). The cell concentration was adjusted to $1–2 \times 10^7$ cells/ml.

The peptide solution (0.1 ml) was added to this cell suspension (0.7 ml) and incubated at 37°C for 30 min. Thereafter, 0.2 ml of the serum from an atopic patient sensitive to dust mites was added to the suspension and the mixture was incubated at 37°C for 120 min. The cells were collected by centrifugation, and washed twice with the standard buffer. The cell suspension ($2–8 \times 10^6$ cells/ml) was prepared with the standard buffer containing 0.6 mM $CaCl_2$ and 1 mM $MgCl_2$. This cell suspension (0.7 ml) was incubated with dust mite extract (Andard-Mount Ltd.) as the allergen at 37°C for 45 min. The mixture was then cooled in an ice-bath and centrifuged. The amount of histamine in the supernatant and precipitate was determined with an automated fluorometer (Technicon Ltd.) by the method of Siraganian [19]. The inhibitory rate of histamine release was evaluated by comparing the amount of histamine released from the cells in the presence of synthetic peptides with that of the control, in which the standard buffer containing 4 mM EDTA and heparin (12.5 μg/ml) was added to the cells instead of the synthetic peptide solution. Three donors were used as each concentration consisted of 3 tubes per donor.

3. RESULTS AND DISCUSSION

In our previous paper [10], we suggested that the synthetic human IgE peptide fragments which inhibited PCA in rats were located within the sequence Ala^{329}–Thr^{357} in the CH3 domain and Arg^{419}–Ala^{463} in the junction between the CH3 and CH4 domains, and that they might be involved in binding of IgE to its receptor. On the other hand, Helm et al. recently suggested that the FcεRI-binding site on human IgE was located within the sequence Gln^{301}–Arg^{376} in the junction between the CH2 and CH3 domains [12,20]. Therefore, we continued the synthesis of the peptide fragments within the sequence Ser^{300}–Lys^{547} of the human IgE molecule to determine the exact site responsible for the IgE–FcεRI interaction. In the synthesis, the cysteine residues of the disulfide bridges in IgE [21], which are assumed not to be involved in the binding between IgE and its receptor, were replaced by serine residues. The possible binding regions to the FcεRI were predicted by secondary structure predictions [22], scans for the hydrophilic [23] and hydrophobic residues [24], and the study of homologous sequences among immunoglobulins. We synthesized various peptides consisting of 4–45 amino acid residues within the sequence 300–547 of human IgE.

To delineate the binding site of human IgE to the FcεRI on human basophils, human basophils were passively sensitized with atopic patient's serum in vitro [17], since the interaction between IgE and FcεRI is relatively specific to species [11]. The acid-treated peripheral basophils of healthy volunteers were passively sensitized with the serum of atopic patient's donor sensitive to dust mites. Histamine release from the cells was induced by the dust mite extract. A quantitative relationship was obtained between the percentage of histamine release and the concentration of its serum. The affinity of myeloma IgE to the FcεRI was comparable to that of native IgE. Myeloma IgE did not recognize the dust mite extract, which was the antigen for the serum of atopic patient's donor. In this test, human myeloma IgE inhibited the passive sensitization in a concentration-related manner at concentrations of 0.3–3 μg/ml (data not shown) [17]. Therefore, this passive sensitization method is capable of revealing whether tested peptides prevent the interaction between human IgE and its receptor.

Table I summarizes the results of the inhibitory activity of the synthetic peptide fragments. Among them, peptide fragments with strong inhibitory activity were found in amino acid sequences 345–356 (compound 11) and 479–496 (compound 26). Furthermore, compound 26 inhibited non-specific histamine release [25] by a Ca^{2+} ionophore, A23187, whereas compound 11 had no inhibitory activity for the non-specific histamine release (data not shown). The amino acid sequence 345–356 (compound 11) was included in a human IgE–FcεRI-binding region reported by Helm et al. [12], and located on the surface of a three-dimensional model [26] of human IgE. In our previous paper [10], compound 11 (Pro^{345}-Phe-Asp-Leu-Phe-Ile-Arg-Lys-Ser-Pro-Thr-Ile^{356}) also inhibited the PCA reaction in rats. Thus, it was suggested that compound 11 is a possible IgE–FcεRI-binding site, thus preventing the interaction between IgE and its receptor on human basophils.

In order to determine the minimal active core, various analogues of compound 11 were prepared and assayed. Shortening the peptide chain of compound 11 (345–356) one by one at its C-terminus gave compounds 41–46 (Table II). As the size of the peptide decreased, inhibitory activity increased, peaking at compound 44 (345–352). Further shortening of the peptide chain decreased the potency. In contrast, the potency of compound 11

Table I

Inhibition of passive sensitization with human IgE by synthetic human IgE peptide fragments in human peripheral basophils in vitro

No.	Compound	Inhibition (%)[a]
1 (300–309)[b]	SQKHWLSDRT	−3.5
2 (305–314)	LSDRTYTSQV[c]	4.3
3 (313–322)	QVTYQGHTFE	−13.8
4 (318–328)	GHTFEDSTKK	−1.8
5 (321–332)	FEDSTKKSADSN[c]	15.6
6 (329–348)	ADSNPRGVSAYLSRPSPFDL	2.1
7 (329–357)	ADSNPRGVSAYLSRPSPFDLFIRKSPTIT	2.4
8 (329–339)	ADSNPRGVSA	1.8
9 (333–344)	PRGVSAYLSRPS	8.9
10 (340–349)	YLSRPSPFDL	4.7
11 (345–356)	PFDLFIRKSPTI	39.7
12 (349–368)	FIRKSPTITSLVVDLAPSKG[c]	−5.7
13 (357–367)	TSLVVDLAPSK[c]	9.2
14 (368–387)	GTVNLTWSRASGKPVNHSTR	−2.2
15 (380–399)	KPVNHSTRKEEKQRNGTLTV	2.4
16 (400–411)	TSTLPVGTRDWI	11.2
17 (411–425)	IEGETYQSRVTHPHL[c]	8.1
18 (417–437)	QSRVTHPHLPRALMRSTTKTS[c]	10.6
19 (419–463)	RVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLA	26.9
20 (434–448)	TKTSGPRAAPEVYAF	−1.1
21 (449–463)	ATPEWPGSRDKRTLA	9.0
22 (454–466)	PGSRDKRTLASLI[c]	0.4
23 (456–461)	SRDKRT	4.7
24 (466–480)	IQNFMPEDISVQWLH	12.3
25 (479–489)	LHNEVQLPDAR	12.8[d]
26 (479–496)	LHNEVQLPDARHSTTQPR	45.1
27 (485–504)	LPDARHSTTQPRKTKGSGFF	−0.4
28 (496–505)	RKTKGSGFFV	6.3
29 (505–510)	VFSRLE	−0.7
30 (510–519)	EVTRAEWEQK	1.0
31 (517–523)	EQKDEFI	−1.2
32 (523–530)	ISRAVHEA[c]	11.9
33 (530–535)	AASPSQ	2.0
34 (531–547)	ASPSQTVQRAVSVNPGK	7.1

[a] Percent inhibition of histamine release at 1×10^{-3} M.
[b] The positions of the synthetic peptide fragments are shown in parentheses. For the residual number, refer to [6].
[c] Cys was replaced with Ser.
[d] Percent inhibition of histamine release at 1×10^{-4} M.

decreased when the same procedure was applied to its N-terminus. Furthermore, extension and shortening of the peptide chain of compound 44 at the N-terminus (compounds 47–52) removed the inhibitory activity, as shown in Table III. Therefore, we conclude that the octapeptide (Pro[345]-Phe-Asp-Leu-Phe-Ile-Arg-Lys[352], compound 44) is the active core of compound 11, in other words, a possible binding site of human IgE.

In an attempt to determine the amino acid residue(s) essential for the inhibitory activity, various analogues, such as omission (compounds 53–60), substitution (by Ala or Gly residue, compounds 61–70), and retro-sequence peptides (compound 71) were synthesized and assayed (Table IV). The potency of the omission analogues of compound 44 was very low relative to compound 44. In a series of single amino acid substitutions by Ala or Gly residues, their activity decreased. In addition, the retro-sequence peptide of compound 44 (com-

Table II

Inhibition of passive sensitization with human IgE by compound 11-related peptides in human peripheral basophils in vitro

No.	Compound	Inhibition (%)[a]	
		1×10^{-4} M	1×10^{-3} M
35	RKSPTI	–	11.9
36	IRKSPTI	–	6.9
37	FIRKSPTI	–	5.0
38	LFIRKSPTI	–	10.4
39	DLFIRKSPTI	–	0.8
40	FDLFIRKSPTI	–	1.0
11	PFDLFIRKSPTI	2.4	39.7
41	PFDLFIRKSPT	12.9	63.3
42	PFDLFIRKSP	22.4	insoluble
43	PFDLFIRKS	28.0	insoluble
44	PFDLFIRK	11.0	78.7
45	PFDLFIR	5.9	insoluble
46	PFDLFI	–	10.6

[a] Percent inhibition of histamine release.

Table III

Inhibition of passive sensitization with human IgE by compound 44-related peptides in human peripheral basophils in vitro

No.	Compound	Inhibition (%)[a]	
		1×10^{-4} M	1×10^{-3} M
47	PSPFDLFIRK	–	12.4
48	SPFDLFIRK	–	10.7
44	PFDLFIRK	11.0	78.7
49	FDLFIRK	–	31.4
50	DLFIRK	–	9.8
51	LFIRK	–	12.8
52	FIRK	–	13.2

[a] Percent inhibition of histamine release.

pound 71) completely removed the activity. These results suggested that the sequence and the type of individual amino acid residue of compound 44 would be essential for the inhibition of histamine release from human basophils by atopic patient's serum.

It can be concluded that the sequence Pro^{345}–Lys^{352} is a possible binding site of IgE, and that the synthetic octapeptide, Pro^{345}-Phe-Asp-Leu-Phe-Ile-Arg-Lys^{352}, inhibited the histamine release from basophils, probably by occupying the Fcε receptor site on the cells.

Table IV

Inhibition of passive sensitization with human IgE by compound 44-related peptides in human peripheral basophils in vitro

No.	Compound[a]	Inhibition (%)[b]		
		1×10^{-5} M	1×10^{-4} M	1×10^{-3} M
44	PFDLFIRK	–	11.0	78.7
53	-FDLFIRK	–	–	31.4
54	P-DLFIRK	–	–	4.7
55	PF-LFIRK	–	–	17.2
56	PFD-FIRK	–	–	12.6
57	PFDL-IRK	–	–	13.5
58	PFDLF-RK	–	–	16.3
59	PFDLFI-K	–	−3.3	insoluble
60	PFDLFIR-	–	5.6	insoluble
61	AFDLFIRK	–	–	41.8
62	PADLFIRK	–	–	8.0
63	PFGLFIRK	–	–	19.8
64	PFDGFIRK	–	–	7.7
65	PFDAFIRK	–	–	7.1
66	PFDLAIRK	–	–	9.1
67	PFDLFGRK	–	–	17.7
68	PFDLFARK	–	–	13.3
69	PFDLFIGK	–	17.0	insoluble
70	PFDLFIRG	0.9	insoluble	insoluble
71	KRIFLDFP	–	–	7.8

[a] A hyphen indicates the deleted position
[b] Percent inhibition of histamine release.

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